Norovirus GII.17 Natural Infections in Rhesus Monkeys, China

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Noroviruses are a leading viral cause of acute gastroenteritis among humans. During the 2014–15 epidemic season, norovirus GII.17 was detected in rhesus monkeys in China. Genetic, structural, and challenge studies revealed virus mutations and verified the infections. Thus, cross-species transmission may occur, and monkeys may be a virus reservoir.

Noroviruses are a leading viral cause of epidemic and sporadic acute gastroenteritis in humans of all ages, causing substantial illness and death. Each year, noroviruses cause ≈21 million infections in the United States and ≈200,000 deaths worldwide. Among the 6 known norovirus genogroups (GI–VI), all GI, most GII, and a few GIV noroviruses infect humans (human noroviruses). Each genogroup includes up to 22 genotypes based on the sequences of major capsid protein 1 (VP1). Although GIV noroviruses were predominant globally for 2 decades, the previously rare GII.17 genotype emerged during the 2014–15 epidemic season in China and other Southeast Asian countries/regions, causing major epidemics of acute gastroenteritis (1,2). Infection of domestic pigs, cattle, dogs, and rhesus macaques with human norovirus has been reported (3–7). We report the detection and characterization of norovirus GII.17 that extensively and naturally infected farm-raised rhesus monkeys in southwestern China.

The Study
In January 2015, a total of 50 fecal samples were randomly collected from the general monkey population at a farm with ≈2,000 monkeys in Kunming City, Yunnan Province, China, in accordance with the guidelines for humane treatment of animals and approved by the Institutional Animal Care and Use Committee of the Institute of Medical Biology at the Chinese Academy of Medical Science. Viral RNA was extracted from 10% fecal suspensions in physiologic saline by use of a QIAGEN Mini RNA kit (Hilden, Germany). We randomly selected 28 RNA samples for calicivirus detection with a 1-step reverse transcription PCR that used the primer pair P289 and P290 (8), designed to amplify a genome fragment encoding the calicivirus RNA-dependent RNA polymerase. One of the samples showed the expected 310-bp calicivirus RNA-dependent RNA polymerase gene fragment, which was confirmed by DNA sequencing. Nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis indicated that this gene fragment was from a GII.17 norovirus, which we named Mk/KM1509/Yunnn/CHN/2015 (monkey GII.17 norovirus).

Next, we amplified and sequenced the full ≈7.5-kb genome of this norovirus. Sequence analysis showed that the monkey GII.17 norovirus genome sequence shared 99% nt identity with the human GII.17 norovirus recently detected in China (2). Phylogenetic analysis among representative full-length VP1-encoding genes revealed 3 clusters of GII.17 noroviruses (A, B, C) (Figure 1) (9). The monkey GII.17 norovirus grouped with cluster C of the recently detected GII.17 human noroviruses in China. To estimate the infection rate of monkey GII.17 norovirus in the monkey population, we designed a new pair of specific primers (199 and 200) based on our newly isolated genome sequence to reanalyze the 50 extracted RNA samples. PCR amplification and DNA sequencing of the PCR products indicated identical GII.17 noroviruses in 16 (32%) samples.

We then performed a challenge experiment to assess infection and replication of this GII.17 norovirus in monkeys. We randomly selected 2 monkeys for which fecal samples were negative for norovirus and intragastrically administered (by nasogastric tube) a GII.17-positive fecal sample (consisting of 1 mL filtered 20% fecal suspension containing 8.3 × 10^4 norovirus genome copies). Despite the absence of typical signs (watery diarrhea and fever), both challenged animals shed norovirus RNA in their feces for at least 16 days; by postinoculation day 3, shedding peaks were 2.573 × 10^7 genome copies/gram feces for 1 monkey and 1.33 × 10^7 for the other (Figure 2, panels A, B). These great increases of the shed genome copies indicated successful infection and replication of the GII.17 norovirus in monkeys.

We also measured possible seroconversion in the challenged animals by using recombinant VP1 protein of the

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monkey GII.17 norovirus developed after the challenge was performed. ELISA with monkey norovirus VP1 as the capture antigen showed that both monkeys had high norovirus IgG titers (1:320) before the challenge. As a result, norovirus-specific antibody titer increases for both challenged animals were only 2-fold (Figure 2, panels C, D). The observed high preexisting norovirus antibody titers in both monkeys selected for challenge may have resulted from previous infection with the GII.17 norovirus, although their fecal samples were norovirus negative by the time of selection for challenge. The observed low antibody responses and the lack of typical clinical signs after norovirus infection via virus challenge may result from relatively high preexisting GII.17 antibody titers. Further study to define the role of preexisting norovirus antibodies in norovirus infection of rhesus monkeys is needed.

Histo-blood group antigens (HBGAs) are norovirus host factors in which hosts with matched HBGAs exhibit increased susceptibility to norovirus infection (10). To improve understanding of the HBGAs binding profile of this monkey GII.17 norovirus, the recombinant VP1 proteins of the new monkey strain and a recent human GII.17 norovirus were expressed in Escherichia coli (online Technical Appendix Figure, panel A), as previously described (11). HBGAs binding assays, performed by using defined human saliva samples with known HBGAs types, revealed that the VP1 protein of the monkey GII.17 norovirus bound to human saliva samples with significantly lower binding signals (optical densities) than the human GII.17 norovirus (online Technical Appendix Figure, panels B and C). Accordingly, sequence comparisons of the P domain (the HBGAs binding domain) between the human and monkey noroviruses and structural analysis based on the known GII.17 P dimer crystal structure (12) revealed 2 residue mutations, D377G and N342S, near the HBGAs binding site of the monkey GII.17 norovirus (online Technical Appendix Figure, panel D). The D377G mutation of the monkey GII.17 norovirus replaces the negatively charged aspartic acid with a small, neutral glycine; the N342S mutation replaces the larger, strongly polar asparagine with a tiny, weakly polar serine. These 2 mutations may be the reason why binding of the monkey GII.17 norovirus to HBGAs is weaker than that of the GII.17 human norovirus. We also noted that the monkey GII.17 VP1 protein bound saliva samples with significantly higher binding signals to saliva samples of type B, which also happens to be the major blood type of rhesus monkeys (13).

Conclusions

Although limited success during monkey challenge studies using human noroviruses has been reported (14,15), our study showed that GII.17 noroviruses were able to infect a monkey population, indicating extensive human norovirus infection of farm-raised rhesus monkeys under natural conditions. Our findings suggest that it may be possible to
establish a useful animal model of norovirus infection to evaluate human norovirus vaccines and antiviral drugs and to study human norovirus pathogenesis, although further testing needs to be done to confirm such possibility. Our findings also raise new concerns about possible viral reservoirs and cross-species transmission of noroviruses.

Considering the fact that a new GII.17 variant emerged as the predominant norovirus and caused major epidemics in China during the same period (1,2), the detected monkey GII.17 norovirus probably originated from a human GII.17 norovirus. However, the mutations near the HBGA binding site might imply an initial adaptation of the monkey GII.17 norovirus to the new host. To provide a better understanding of its infection, pathogenesis, host specificity, epidemiology, and cross-species transmission, further characterization of this monkey GII.17 norovirus is warranted. This information may also be valuable for the future establishment of a monkey model of norovirus infection for vaccine and antiviral evaluation and for addressing the concerns of unknown viral reservoirs and potential zoonotic infection of noroviruses.

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Technical Appendix

Technical Appendix Figure. Production and characterization of the monkey and human GII.17 norovirus (NoV) recombinant viral capsid protein (VP) 1. A) Expression of the human (left) and monkey (right) GII.17 NoV recombinant VP1 proteins. The human GII.17 NoV was the most recent variant (DG42) isolated from a NoV outbreak (1). B and C) The binding signal intensities (optical densities [ODs], y-axis) of human (B) and monkey (C) GII.17 VP1s to the same set of saliva samples (x-axis), with defined histo-blood group antigen (HBGA) types, through saliva-based HBGA binding assays. The human saliva samples were collected from healthy volunteers in Ohio, USA, with defined type O, A, B, and nonsecretor (N) types. VP1 proteins at 3 indicated concentrations were used for the assays. D) The GII.17 P dimer
crystal structure (PDB code 5F4O). The HBGA binding sites are shown in orange; 3 surface mutations (N342S, D377G, and Y505H) are shown in green and amino acids are indicated.

Reference

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